

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Molecular Features of Virulence and Resistance Mechanisms in Nosocomial and Community-Acquired *Staphylococcus aureus*

---

Irina Gheorghe, Marcela Popa and  
Luminița Gabriela Măruțescu

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.75191>

---

## Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are known for their emergent multi-drug resistance phenotypes, implication in nosocomial infections and outbreaks worldwide, being commonly associated with hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) skin and soft tissue infections. *S. aureus* causes a wide spectrum of clinical symptoms, ranging from mild to life-threatening diseases; disease severity is determined by microorganism-related virulence factors and host condition. The ability of these strains to form microbial biofilms, one of the main pathogenicity factors, generates difficult medical problems, favored by the widespread use of large invasive medical procedures (probes, catheters, heart valves, prostheses). Contamination of these devices is associated with the risk of subsequent development of human infections. The knowledge of virulence and antibiotic resistance patterns of HA-MRSA and CA-MRSA and encoding genes are very important for supporting effective infection control measures and therapy of staphylococcal infections.

**Keywords:** *Staphylococcus aureus*, MRSA, resistance, virulence, biofilm

---

## 1. Introduction

Staphylococci are commensal bacteria that form part of microbiota of human and animal skin and mucous membranes. Among more than 40 species of the genus, *Staphylococcus aureus* is colonizing the nostrils and skin of ~30% of the population [1, 2]. *S. aureus* is an opportunistic pathogen,

causing infections when it crosses the barriers of natural defense and escapes the mechanisms of anti-infectious protection. Factors favoring staphylococcal infections include local (lesions of the skin, the presence of implants, catheters, etc.) and general (innate or acquired deficiencies of the immune system such as complement system deficiencies, granulocytopenia, agranulocytosis, AIDS, diabetes, immunosuppressive treatments, etc.). It is able to cause a plethora of community (CA) and health care (HA) infections, ranging from superficial skin infections to severe, and potentially fatal, invasive diseases [3–5] due to its ability to produce a spectrum of virulence factors and resistance to multiple antibiotics, frequently encoded by mobile genetic elements (MGEs) [6], which have eased the persistence of *S. aureus* in hospital environment [7]. *S. aureus* is causing skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis [8]. *S. aureus* is the most commonly isolated bacteria from wound infections and studies involving patients with chronic venous leg ulcers found *S. aureus* positive cultures in 88–93.5% of infections [9]. Bacteremia caused by *S. aureus* is associated with higher morbidity and mortality, compared with bacteremia caused by other pathogens, with an annual incidence rate of 20–50 cases/100,000 population, and a mortality rate of 10 and 30%. The highest mortality rates occur in patients with primary bacteremic pulmonary infections and infective endocarditis, whereas the lowest rates occur in patients with central or peripheral venous catheter-related infections [10]. The major concern refers mainly to methicillin-resistant *S. aureus* (MRSA) isolates. Health care-associated MRSA (HA-MRSA) are represented by the *S. aureus* strains isolated from patients after a hospitalization of two or more days or with the MRSA risk factors (history of recent hospitalization, surgery, dialysis, catheters, etc.). Community-associated MRSA (CA-MRSA) are those *S. aureus* isolates obtained from patients within 2 days of hospitalization and without the above-mentioned MRSA risk factors [11]. Infections by CA-MRSA isolates are usually associated with children, young age, recurrent infections and the use of injectable drugs [12].

In this chapter, we review some aspects related to resistance and virulence features in CA-MRSA and HA-MRSA strains, underlying the evolution of the highly successful community- and health care-associated lineages and their plasticity in ability to adapt to environmental changes.

## 2. *Staphylococcus aureus* resistance to antibiotics

Antistaphylococcal antibiotics are mainly targeting cell wall synthesis, proteins and nucleic acid synthesis, and different metabolic pathways. The large use of antibiotics, not only in the medical field but also in the agriculture has facilitated the evolution and spread of resistance genes [13]. Bacterial resistance can be constitutive (mutations of the target genes, efflux pumps overexpression, etc.) or acquired by horizontal gene transfer via various mobile genetic elements like plasmids, transposons, bacteriophages, pathogenicity islands, and staphylococcal cassette chromosomes [14]. Plasmids and staphylococcal cassette chromosomes in particular have played a central role in conferring resistance to  $\beta$ -lactam antibiotics and vancomycin [15].

Penicillin resistance is conferred by  $\beta$ -lactamase, an extracellular enzyme encoded by *blaZ* that is active when bacteria are exposed to  $\beta$ -lactam antibiotics. The enzyme acts on  $\beta$ -lactam ring by opening it through hydrolization [16].

Methicillin resistance requires the presence of the chromosomally localized *mecA* gene. The *mecA* gene and its regulatory elements, form the *mec* complex: SCC*mec* elements carry the *mecR1* and *mecI* genes, which regulate the expression of *mecA*, with increased *mecA* translation induced by  $\beta$ -lactam antibiotic exposure. SCC*mec* elements can also carry resistance genes for other antibiotics and heavy metals as well as the *psm-mec* locus, which encodes cytotoxin termed phenol-soluble modulins-mec (PSM-mec) [16].

PBPs are membrane-bound enzymes that are used in cross-linkage of peptidoglycan chains by catalyzing the transpeptidation reaction [17]. PBP2a has a low affinity for  $\beta$ -lactam antibiotics and thus methicillin resistance also grants resistance to all  $\beta$ -lactam antibiotics [16].

Eleven types of SCC*mec* have been described, distinguished by the type of *ccr* gene complex that mediates the site-specific excision and insertion of the SCC*mec* cassette out of or into the bacterial genome and the class of *mec* complex that they bear [18].

There have been revealed molecular differences between CA-MRSA and HA-MRSA strains regarding the types of SCC*mec*: HA-MRSA strains carry the large staphylococcal chromosomal cassette *mec* (SCC*mec*) belonging to type I–III and containing the *mecA* gene, mostly universal among MRSA isolates and usually are resistant to several classes of non- $\beta$ -lactam antibiotics. It seems that the large SCC*mec* types I–III are present in HA-MRSA strains and were transferred to *S. aureus* from a commensal staphylococcal species [19]. Carriage of the *psm-mec* locus from type II SCC*mec* elements attenuates virulence, suppresses colony spreading activity, reduces expression of the chromosomally encoded PSMa, and promotes biofilm formation [20].

HA-MRSA strains seldom carry the genes for the Panton-Valentine leukocidin (PVL). CA-MRSA isolates carry smaller SCC*mec* elements, most commonly SCC*mec* type IV or type V [21]. CA-MRSA strains are resistant to fewer non- $\beta$ -lactam classes of antibiotics and frequently carry PVL genes. There has been suggested that the smaller SCC*mec* types IV, V, VI, and VII have been transferred to methicillin-susceptible backgrounds [21, 22]. One study suggested that the type IV SCC*mec* element has been transferred to an MSSA strain [23]. The type IV SCC*mec* was originally associated with MRSA infections in patients with no HA-MRSA risk factors [21].

There have been revealed that the deletion of the gene encoding PBP 4 in two common CA-MRSA isolates, USA300 and USA400, resulted in a 16-fold reduction in oxacillin and nafcillin resistance in these particular strains. These studies suggest that PBP 4 is a significant target for the discovery of agents effective against CA-MRSA [24].

There have been also reported CA-MRSA strains positive for *mecA* and PBP 2a that were phenotypically oxacillin susceptible [25]. It was therefore suggested that *mecA* expression alone does not appear to be sufficient to guarantee phenotypic methicillin resistance, and that the existence of additional molecular targets could be associated with the susceptibility of oxacillin in certain strains and the involvement of genes different from the known effectors of methicillin resistance in CA-MRSA. The *vraS/vraR* two-component regulatory system is required for oxacillin resistance in CA-MRSA [26, 27].

The emergence/re-emergence of successful *S. aureus* clones suggests a rapid bacterial adaptation and evolution, which includes the emergence of antibiotic resistance and increased virulence and/or transmissibility.

Most of the nosocomial MRSA infections are caused by five major lineages that circulate internationally: CC5, CC8, CC22, CC45 and CC30 [28].

ST1 pulsotype USA 400—represented the most frequently CA-MRSA clone in the United States after 1990s; was characterized by carrying SCC<sub>mec</sub> type IV that has been usually susceptible to most non- $\beta$ -lactam antibiotics and cause SSTIs. This strain lacked PVL genes and circulates in the community in Australia (WA-MRSA-1) and England [18, 29]. ST80 is PVL+-bearing SCC<sub>mec</sub> type IV and have been reported in several Western European countries such as Austria, Norway, Denmark, Sweden, England, Switzerland, Greece and France [18]. ST30 corresponds to phage type 80/81 nosocomial strain of *S. aureus* from United States during the 1950s and 1960s. These strains were MSSA strains that carried the PVL genes [30] and the SCC<sub>mec</sub> type IV element. ST59 strains are PVL+, they have diverse spa types and several SCC<sub>mec</sub> types were isolated from different countries worldwide like: Taiwan, Australia, Denmark, Netherlands England, and the United States [18]. In Taiwan, ST59 clones with a multidrug-resistant phenotype are the most encountered, having a distinctive SCC<sub>mec</sub> DNA sequence [31]. ST93 was first identified in 2000 in Queensland and New South Wales, Australia. It spread rapidly to become the predominant PVL+ MRSA clone isolated from infections in those regions. USA300 Strains has the following characteristics: the carriage of SCC<sub>mec</sub> type IV, PVL genes, and, in most strains, the ACME element. USA300 is classified as ST8 by MLST and is usually classified as t008 by spa typing. It is frequently susceptible to several non- $\beta$ -lactam antibiotics and became the dominant CA-MRSA strains in the United States, has been also identified in Western Europe [18], Japan [32] and Australia, where it has been called WA-MRSA-12 [18, 33].

MRSA infections in the community can also be caused by livestock-associated MRSA (LA-MRSA). LA-MRSA is initially associated with livestock and differs from genotypic HA-MRSA and genotypic CA-MRSA in its genomic traits. CC9 clonal complex (LA-MRSA) is most frequent among livestock in Asia [34]. In the USA, ST5 (MRSA) was isolated from pigs [35]. CC1 (MRSA) is prevalent in Romanian nosocomial infections and has low host specificity [36].

The quinolones act on DNA gyrase, which relieves DNA supercoiling, and topoisomerase IV, which separates concatenated DNA strands. Resistance to quinolones results from the stepwise acquisition of chromosomal mutations [16]. Quinolone affinity is reduced by changes of the amino acids in the enzyme-DNA complex (quinolone resistance-determining region [QRDR]). The most common sites of resistance mutations are GyrA subunit in gyrase and ParC (GrlA in *S. aureus*) of topoisomerase IV. Drugs primarily target topoisomerase IV and the mutations at this level are essential for resistance [37, 38]. The confluence of high bacterial density, especially inside biofilms, the likely preexistence of resistant subpopulations, and the sometimes limited quinolone concentrations achieved at sites of staphylococcal infections creates an environment that fosters selection of resistant mutants [37].

An additional mechanism of resistance in *S. aureus* is induction of the NorA multidrug-resistance efflux pump with increased expression can result in low-level quinolone resistance [39].

*S. aureus* response to vancomycin inhibitory activity divides the strains into sensitive, intermediate and resistant. Vancomycin intermediate-resistant *S. aureus* (VISA) have MICs to vancomycin of 8–16  $\mu\text{g/ml}$  (MIC = 4–8  $\mu\text{g/ml}$ ). There has been identified the existence of a pre-VISA stage of resistance known as heterogeneous VISA (hVISA) [16]. An hVISA phenotype



refers to a mixed cell population—derived originally from a single colony of *S. aureus*—in which the majority of cells have little or no resistance to vancomycin ( $\text{MIC} \leq 2 \mu\text{g/ml}$ ) and a sub-population of cells is resistant to the antibiotic at the level of VISA ( $\text{MIC} \geq 4 \mu\text{g/ml}$ ) [40].

The molecular mechanisms that underlie development of hVISA are incompletely defined. Fundamental characteristics of the VISA phenotype include increased cell wall thickness, caused by differentially regulated cell wall biosynthesis and stimulatory pathways [15, 41], reduced cross-linking of peptidoglycan, decreased autolytic activity of the enzymes responsible to cell-wall turnover [15, 42], altered surface protein profile, dysfunction of the *agr* system and changes to growth characteristics [15, 43].

Molecular basis of the VISA phenotype is not fully understood but several genes/mutations are known to contribute to its development. The mutations within genes encoding two-component regulatory systems, such as *graRS* and *walKR* are of particular significance. GraRS differentially regulates transcription of cell wall biosynthesis genes and has been associated with a broad array of genes and regulators that play a role in the intermediate resistance phenotype [44]; GraRS also up-regulates genes in the capsule biosynthesis operon, leading to increased capsule production [44]. GraRS up-regulates the *dlt* operon and the *mprF/fmtC* genes, which are linked to teichoic acid alanylation and alteration of cell wall charge [15]. Point mutations within *graRS* reduced susceptibility to vancomycin [15, 45] and *graRS* mutations are linked to modified expression of global regulators, *rot* and *agr* [15, 44]. *rpoB* a gene encoding the DNA-dependent RNA polymerase  $\beta$ -subunit is commonly associated with increased resistance to vancomycin, prolonged propagation time and increased cell wall thickness [15, 46]. VISA isolates have been shown to have non-silent mutations in *vraSR*. Such mutations could lead to downstream up-regulation of over 40 cell wall synthesis genes, including genes required for producing cell wall derivatives such as D-Ala-D-Ala [15].

The VISA strains produce considerable amounts of peptidoglycan and this generates thicker, irregularly shaped cell walls. They also expose more D-Ala-D-Ala residues available to bind and trap vancomycin which acts as a further impediment to drug molecules reaching their target on the cytoplasmic membrane [16].

Complete vancomycin resistance in *S. aureus* ( $\text{MIC} \geq 16 \mu\text{g/ml}$ ) is conferred by the *vanA* operon (containing *vanA*, *vanH*, *vanX*, *vanS*, *vanR*, *vanY* and *vanZ* genes) encoded on transposon Tn1546, originally a part of a vancomycin-resistant enterococci (VRE) conjugative plasmid [15, 47]. The *vanA* operon is controlled via a two-component sensor-regulator system encoded by *vanS* and *vanR* that sense vancomycin and activate transcription of the operon, respectively, VanA, VanH and VanX together are essential for the vancomycin resistance phenotype. *S. aureus* can acquire enterococcal plasmids during discrete conjugation events. Vancomycin resistance in *S. aureus* is maintained by retaining an original enterococcal plasmid or by a transposition of Tn1546 from the VRE plasmid into a staphylococcal resident plasmid [15, 48].

### 3. *Staphylococcus aureus* adhesion and biofilm development

The broad range of infections caused by *S. aureus* is related to a number of virulence factors that allow it to adhere to surface, invade or avoid the immune system and cause harmful toxic effects to the host [49].

The initialization of the colonization process is started by the attachment of *S. aureus* to the host cell surface through adhesins. Proteins covalently anchored to cell peptidoglycans represent one major class of *S. aureus* adhesins, which attach to the extracellular matrix or plasma components via a threonine residue and are known as the microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) [49].

Staphylococcal cell wall-anchored (CWA) proteins are secreted by the Sec system and share a C-terminal cell wall anchoring motif, hydrophobic domain and positively charged domain [50]. Foster et al. [50] proposed to classify the Staphylococcal CWA proteins into four groups based on structural motifs: MSCRAMMs (microbial surface component recognizing adhesive matrix molecules), the NEAT motif family, the three-helical bundle family and the G5-E repeat family. All of these types of CWA proteins are involved in staphylococcal biofilm formation. MSCRAMMs are adhesins that contain at least two IgG-like folds and employ a ligand binding mechanism called dock, lock and latch [50]. MSCRAMMs are composed of a binding domain, a cell wall spanning domain and a domain for the covalent or non-covalent attachment. These adhesins can bind one or more human proteins (collagen—mostly via Cna, fibronectin—via FnbAB, fibrinogen—via ClfAB and Fib) [51, 52].

The Staphylococcal MSCRAMMs are the Clf-Sdr family proteins, including bone sialoprotein-binding protein (Bbp), the fibronectin-binding proteins (FnBPs) and collagen adhesion (CNA) [53]. The Clf-Sdr family consists of Clumping factor A (ClfA), clumping factor B (ClfB) and the Sdr proteins. ClfA and ClfB are fibrinogen-binding proteins in *S. aureus* [50, 53]. Rot and agr affect bacterial binding to fibrinogen by regulating clfB but not clfA [54]. *S. aureus* has two fibronectin-binding proteins, FnBPA and FnBPB, encoded by fnbA and fnbB, respectively. FnBP binding to fibronectin induces bacterial invasion into epithelial cells, endothelial cells and keratinocytes [53]. The FnBPs affect biofilm formation by a self-association mechanism that is distinct from ligand binding and virulence [55, 56].

The NEAT motif family consists of the iron-regulated surface determinant (Isd) proteins who bind heme or hemoglobin, facilitating its transport into the bacterial cell, and they are up-regulated in iron-limiting conditions [57]. *S. aureus* IsdA is the most abundant CWA protein in iron starvation conditions, and also decreases surface hydrophobicity, which makes *S. aureus* more resistant to bactericidal fatty acids and peptides in human skin. IsdA also is able to bind human fibrinogen and fibronectin [53].

The sole three-helical bundle cell wall-anchored protein is Staphylococcal Protein A (SpA), which is present in all strains of *S. aureus*. SpA allows immune evasion by binding to the conserved Fc region of immunoglobulin IgG, and contributes to disruption of the host immune response by promoting bacterial survival in human blood after being released from the cell wall [53, 58].

**G5-E Repeat Family:** Aap/SasG—G5-E repeats are found in cell wall-anchored adhesins in Gram-positive organisms (are named after the five conserved glycine residues in each repeat). *S. aureus* SasG promotes attachment to human desquamated nasal epithelial cells via its A domain [59]. Multiple studies have shown that the G5-E repeats of SasG and Aap are able to dimerize by binding to  $Zn^{2+}$ , forming a “twisted rope” structure [53, 60]. This property is thought to enable intercellular adhesion when adjacent SasG or Aap proteins dimerize via their G5-E domains.

Uncategorized CWA Proteins—the remaining uncategorized cell wall-anchored proteins are Bap and several Sas proteins, including SasA/SraP. SasX is another cell wall-anchored adhesin that has been shown to play an important role in virulence [53].

Surface-associated proteinaceous adhesins—autolysins AtlA and AtlE are found in *S. aureus* involved in cell wall turnover, cell division and cell lysis [53], they attach to extracellular matrix materials and can augment the biofilm matrix with eDNA by inducing cell lysis.

Non-proteinaceous surface-associated adhesins—wall teichoic acids and lipoteichoic acids have been shown to play a role in adhesion, colonization of host cells and biofilm formation. Wall teichoic acids are covalently linked to the peptidoglycan and consist of alternating phosphate and ribitol, while lipoteichoic acids attach to the outer leaflet of the cell membrane and have alternating phosphate and glycerol [61].

The polysaccharide intercellular adhesion (PIA) is a secreted polysaccharide that is synthesized by the *ica* operon and has been thoroughly studied in the context of biofilm formation, immune evasion, and pathogenesis. PIA is a glycan of  $\beta$ -1,6-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues with a net positive charge that promotes intercellular aggregation and attachment of cells to inert surfaces. The *ica* operon consists of four biosynthesis genes such as *icaA*, *icaD*, *icaB* and *icaC* and a divergently transcribed repressor, *icaR*. Carriage of the *ica* locus is a characteristic of most clinical *S. aureus* strains [62].

One of the reasons the staphylococcal infections are difficult to eradicate is the bacteria's ability to develop community structures known as biofilms by attaching to different surfaces (tissues, catheters and medical devices), and often occur in areas of the body that are not easily accessible for treatment [63].

Biofilm is an assemblage of microbial cells that are irreversibly associated to a surface and embedded in a protective extracellular polymeric matrix. Biofilm-associated organisms have proteins production and genes expression modification compared to their planktonic counterparts [64].

Biofilm formation can be divided into three major stages: initial attachment, development/maturation of the biofilm and dispersion.

During initial attachment, bacteria adhere to the available surface and in case of abiotic one its conditioning is important through various physiochemical parameters: chemical composition of the material, hydrophobicity, electrostatic charges, surface energy and surface roughness and in the case of biotic adhesion: serum and tissue protein adsorption [65].

The final stage of biofilm development is the detachment of cells from the biofilm colony and their dispersal into the environment, which contributes to biological dispersal, bacterial survival and disease transmission. Like other stages of biofilm development, dispersal is a complex process that involves numerous environmental signals, signal transduction pathways and effectors [66].

The biofilm matrix is a complex structure that contains extracellular DNA (eDNA), both from lysed bacteria and potentially from host neutrophil cell death [53], proteinaceous adhesins directly associated with bacteria in the biofilm or free in the biofilm matrix [67], recycled



cytoplasmic proteins that moonlight as components of the extracellular matrix [68], the extracellular polysaccharide intercellular adhesin (PIA), teichoic acids [53]. The matrix can impede the access of certain types of immune defenses, such as macrophages [69].

Cell adhesion and subsequently biofilm formation are processes mediated by covalently and non-covalently cell wall proteins and non-protein factors. For *S. aureus* more than 20 adhesins were identified [53].

Switching between planktonic and biofilm-forming modes represents a major life style change for microbes, and has been shown to be a tightly regulated process [70] through quorum sensing (QS) which is a cell-cell communication mechanism in which bacteria secrete and sense small diffusible molecules called autoinducers (AIs) to coordinate social activities, such as bioluminescence, biofilm formation, swarming behavior, antibiotic production and virulence factor secretion [71].

Staphylococcal biofilm formation is affected by growth conditions (e.g., NaCl, glucose, human plasma, etc.) and is controlled by multiple global regulators such as SarA, Agr, SigB and Sae. The Sae-regulon includes both the factors promoting biofilm formation (i.e., Coa, Emp, Eap, FnBPA, FnBPB, Hla and Hlb) and biofilm dispersal factors (nuclease and proteases) and depending on growth conditions and strain backgrounds, the Sae system could affect biofilm formation either positively or negatively [72].

Bacteria in biofilms can tolerate ten to thousand fold higher levels of antibiotics than the genetically equivalent planktonic bacteria. Staphylococcal biofilms cause biomaterial-associated infections which do not respond to antimicrobial treatment often requiring removal of the same leading to substantial morbidity and mortality. It has also been observed that biofilms harbour persister cells and small colony variants [73], whereas planktonic persisters are eliminated by the immune system *in vivo*, persisters in biofilms serve as a shield evading the immune response and a reservoir of such shielded persisters is a potential source for the emergence of heritable antibiotic resistance [14].

#### 4. *Staphylococcus aureus* soluble virulence factors

*S. aureus* secretes numerous exotoxins, including polypeptides that destroy the integrity of the host cell plasma membrane. These polypeptides are pore-forming toxins (PFT):  $\alpha$ -hemolysin and the bi-component leukocidins  $\gamma$ -hemolysin,  $\beta$ -hemolysin, the Pantone Valentine leukocidin (PVL), LukED, and LukGH/AB and phenol soluble modulins (PSMs) [74].

$\alpha$ -Hemolysin is the most characterized virulence factor of *S. aureus*. Upon binding to the cell surface,  $\alpha$ -hemolysin forms pores that allow the transport of molecules such as  $K^+$  and  $Ca^{2+}$  ions, leading to necrotic death of the target cell.

*S. aureus* possesses several other PFTs in addition to  $\alpha$ -hemolysin that for pore formation involves two polypeptides that have been named S (slow) and F (fast) based on their electrophoretic mobility. The PFTs include (i)  $\gamma$ -hemolysin corresponding to two combinations of a

S component (HlgA or HlgC) with a F component (HlgB); (ii) the PVL made of LukS-PV and LukF-PV; (iii) LukED and (iv) LukGH, also known as LukAB [74]

The  $\gamma$  hemolysin variant and leukocidin E-D gene, as well as other genes encoding exotoxins, were detected evenly in HA and CA-MRSA strains, while *sec* and *sek* genes were found only in CA-MRSA strains [75].

$\beta$ -Hemolysin is a neutral sphingomyelinase, it hydrolyses a plasma membrane lipid—sphingomyelin and does not form pores in the plasma cell.  $\beta$ -hemolysin's enzymatic activity is required for its hemolytic activity [74].

$\delta$ -Hemolysin is a small amphipathic  $\alpha$ -helix-structured peptide (26 AA). Its hemolytic activity can be realized by forming transmembrane pores, affecting the membrane curvature or acting as detergent to solubilize the membrane [76].

This family of cytotoxic peptides includes new peptides termed PSM. PSMs represent a secreted  $\alpha$ -helical peptides produced by different *Staphylococcus* species. PSMs encoding genes are able to activate and lyse human neutrophils and are generated at high concentrations by CA-MRSA strains [77]. The *psm-mec*, was the first PSMs gene found within an SCCmec MGE and was linked to the class A *mec* gene complex present in SCCmec types II, III and VIII, with a conserved location next to the *mecI* gene [74, 78].

Protein A prevents the opsonization and phagocytosis by ineffectually binding the Fc region of IgG. It also initiates a proinflammatory cascade in the airway by activating tumor necrosis factor receptor 1 (TNFR1) and B cells in concert with other ligands. MRSA strains with certain *spa* types have a decreased ability to invade human cells in vitro, revealing an association with certain *spa* types and virulence [18].

*S. aureus* produces a group of toxins called the toxic shock syndrome toxin-1 (TSST-1) and enterotoxins (SEA, SEB, SECn, SED, SEE, SEG, SEH and SEI) and the exfoliative toxins A and B (involved in staphylococcal scalded skin syndrome). Cytolytic toxins form pores of holes called  $\beta$ -barrel pores in the plasma membrane. This leads to leakage of the cell's content and lysis of the target cell [79].

PVL is a bi-component exotoxin transmitted by bacteriophages, encoded by two genes, *lukF-PV* and *lukSPV*. PVL genes are carried by CA-MRSA strains and in a small proportion by clinical MSSA strains. Therefore PVL plays an important role in fitness, transmissibility and virulence, but the role of PVL in the pathogenesis of CA-MRSA infections is not fully understood [80]. PVL genes are spreading among *S. aureus* strains by clonal expansion and horizontal transfer. There have been demonstrated that  $\beta$ -lactam antibiotics increased the production of PVL in vitro through transcriptional activation [81, 82]. PVL inserts itself into the host's plasma membrane and forms a pore of a hole. PVL exhibits a high affinity toward leukocytes. PVL can inactivate mitochondria and induce apoptosis. In animal models, PVL has been shown to be dermonecrotic [18], perhaps explaining the pathobiology of the characteristic skin lesions associated with CA-MRSA SSTIs. That the presence/absence of PVL genes in MRSA strains did not interfere with strain virulence in mouse models of sepsis and SSTI, and their presence did not decrease neutrophil survival in in vitro assays [18].

$\alpha$ -Toxin lyses immune cells like macrophages and lymphocytes, alters platelet morphology, which increased thrombotic events associated with *S. aureus* sepsis [83]. The nucleases, proteases, lipases, hyaluronidase and collagenase convert local host tissue into nutrients required for bacterial growth.

Arginine catabolic mobile element (ACME) is a large MGE that plays an important role in the growth, transmission and pathogenesis of CA-MRSA. Two main gene clusters identified as *arc* genes (*arcA*, *arcB*, *arcC* and *arcD*) and the *opp* genes (*opp-3A*, *opp-3B*, *opp-3C*, *opp3-D* and *opp3-E*) are recognized to be virulence factors [84].

## 5. Regulation of virulence factors expression in *Staphylococcus aureus*

In *S. aureus*, virulence factors production is coordinated by different regulators, for example, DNA binding proteins (e.g., SarA and its homologues), two-component signaling systems (e.g., ArlRS, AgrAC, SaeRS and SrrAB) and alternative sigma factor B [72].

Among the regulatory elements, the Agr (the accessory gene regulator) system is the only characterized QS system in *S. aureus* and controls the expression of approximately 150 genes [85]. Agr system regulation depends on cell density. During initial stage of colonization, when there is a low cell density, the Agr QS system is expressed low, but when the biofilm reach maturation and the cell density is high, Agr activity increases and upregulates secreted virulence factors. The *agr* regulation of the proteases is via Rot, whose transcriptional repression of the proteases is relieved when *agr* is induced [65, 86].

*S. aureus* produces AI-2 through the functional *luxS* gene it has. Due to the dual function of LuxS and the absence of genomic evidence of established AI-2 receptors, the AI-2 quorum-sensing function in *S. aureus* has been intangible, until now [71].

Sigma B (SigB) is an alternative sigma factor of RNA polymerase that is activated in stress response and modifies gene expression. SigB upregulates the expression of different factors involved in initial stages of biofilm formation like coagulase, FnBPA and clumping factor [87]. It also controls negative factors that are associated with a planktonic phenotype and seeding dispersal, including enterotoxin B, cysteine protease (SplB), serine protease (SplA), the metalloprotease Aur, staphopain, leukotoxin D and  $\beta$ -hemolysin [53].

The *sar* (Staphylococcal accessory regulator) locus produces three transcripts from three separate promoters, all of which contain the ORF for the DNA-binding protein SarA [53]. SarA also directly regulates several genes that affect biofilm formation. There have been demonstrated that the mutation of *sarA* gene in the USA300 clone limits accumulation of  $\alpha$ -toxin and PSMs through the increased production of extracellular proteases rather than from transcription of the *hla* or *agr* genes [88].

## 6. Conclusions

*Staphylococcus* spp. are common residents of the normal human and animal microbiota, but in certain favoring conditions, they can surpass the anti-infectious defense barriers and become

opportunistic pathogens. Staphylococci can cause numerous types of infections some of them potentially fatal due to their numerous virulence factors, biofilm formation capacity and antibiotic resistance mechanisms. Taking into account the increased incidence and spread of MRSA and multiple-drug resistant strains, a better knowledge of the virulence and pathogenicity mechanisms and of their relationships with resistance, as well as of the quorum sensing mechanisms is essential for the development of novel anti-staphylococcal strategies, targeting the expression of virulence factors or of their regulatory mechanisms.

## Acknowledgements

We thank Professor Mariana Carmen Chifiriuc for the kind guidance and supervision.

## Conflict of interest

There is no conflict of interest.

## Author details

Irina Gheorghe<sup>1,2</sup>, Marcela Popa<sup>1,2\*</sup> and Luminița Gabriela Măruțescu<sup>1,2</sup>

\*Address all correspondence to: [bmarcelica@yahoo.com](mailto:bmarcelica@yahoo.com)

1 Faculty of Biology, University of Bucharest, Bucharest, Romania

2 Research Institute of the University of Bucharest, ICUB Life, Environmental and Earth Sciences, Bucharest, Romania

## References

- [1] Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, Jensen BJ, Killgore G, Tenover FC, Kuehnert MJ. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. The Journal of Infectious Diseases. 2008;**197**:1226-1234. DOI: 10.1086/533494
- [2] Miller LG, Diep BA. Clinical practice: Colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. 2008;**46**:752-760. DOI: 10.1086/526773
- [3] Sergelidis D, Angelidis AS. Methicillin-resistant *Staphylococcus aureus*: A controversial food-borne pathogen. Letters in Applied Microbiology. 2017;**64**:409-418. DOI: 10.1111/lam.12735

- [4] Horn J, Stelzner K, Rudel T, Fraunholz M. Inside job: *Staphylococcus aureus* host-pathogen interactions. International Journal of Medical Microbiology. DOI: 10.1016/j.ijmm.2017.11.009
- [5] Olaniyi R, Pozzi C, Grimaldi L, Bagnoli F. *Staphylococcus aureus*-associated skin and soft tissue infections: Anatomical localization, epidemiology, therapy and potential prophylaxis. In: Current Topics in Microbiology and Immunology. Berlin, Heidelberg: Springer; 2016. DOI: 10.1007/82\_2016\_32
- [6] Malachowa N, Deleo FR. Mobile genetic elements of *Staphylococcus aureus*. Cellular and Molecular Life Sciences. 2010;**67**:3057-3071. DOI: 10.1007/s00018-010-0389-4
- [7] Jani M, Sengupta S, Hu K, Azad RK. Deciphering pathogenicity and antibiotic resistance islands in methicillin-resistant *Staphylococcus aureus* genomes. Open Biology. 2017;**7**(12). DOI: 10.1098/rsob.170094
- [8] Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG. *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews. 2015;**28**(3):603-661. DOI: 10.1128/CMR.00134-14
- [9] Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: Properties, regulation, and roles in human disease. Virulence. 2011;**2**:445-459. DOI: 10.4161/viru.2.5.17724
- [10] Van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in *Staphylococcus aureus* Bacteremia. Clinical Microbiology Reviews. 2012;**25**(2):362-386. DOI: 10.1128/CMR.05022-11
- [11] Gnanamani A, Hariharan P, Maneesh PS. *Staphylococcus aureus*: Overview of bacteriology, clinical diseases, epidemiology, antibiotic resistance and therapeutic approach. In: Enany S, Crotty Alexander LE, editors. Frontiers in *Staphylococcus aureus*. Rijeka: Intech; 2017. DOI: 10.5772/67338.ch1
- [12] Neapolitano LM. Early appropriate parenteral antimicrobial treatment of complicated skin and soft tissue infections caused by MRSA. Surgical Infections. 2008;**9**(1):S17-S27. DOI: 10.1089/sur.2008.063.supp
- [13] Allen HK, Donato J, Wang HH, Cloud-hansen KA, Davies J, Handelsman J. Call of the wild: Antibiotic resistance genes in natural environments. Nature Reviews. Microbiology. 2010;**8**:251-259. DOI: 10.1038/nrmicro2312
- [14] Choudhury R, Panda S, Sharma S, Singh DV. In: Pana M, editor. Staphylococcal infection, antibiotic resistance and therapeutics, Antibiotic Resistant Bacteria — A Continuous Challenge in the New Millennium. Rijeka: InTech; 2012. DOI: 10.5772/29395.ch10
- [15] McGuinness WA, Malachowa N, DeLeo FR. Vancomycin resistance in *Staphylococcus aureus*. The Yale Journal of Biology and Medicine. 2017;**90**(2):269-281
- [16] Lowy FD. Antimicrobial resistance: The example of *Staphylococcus aureus*. Journal of Clinical Investigation. 2003;**111**(9):1265-1273. DOI: 10.1172/JCI200318535
- [17] Ghuysen JM. Molecular structures of penicillin-binding proteins and beta-lactamases. Trends in Microbiology. 1994;**2**:372-380



- [18] David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews*. 2010;**23**(3):616-687. DOI: 10.1128/CMR.00081-09
- [19] Ma XX, Ito T, Tiensasitorn C, Jamklang M, Chongtrakool P, Boyle-Vavra S, Daum RS, Hiramatsu K. Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrobial Agents and Chemotherapy*. 2002;**46**:1147-1152. DOI: 10.1128/AAC.46.4.1147-1152.2002
- [20] Pozzi C, Waters EM, Rudkin JK, Schaeffer CR, Lohan AJ, Tong P, Loftus BJ, Pier GB, Pd F, Massey RC, O'Gara JP. Methicillin resistance alters the biofilm phenotype and attenuates virulence in *Staphylococcus aureus* device-associated infections. *PLoS Pathogens*. 2012;**8**(4):e1002626. DOI: 10.1371/journal.ppat.1002626
- [21] Berglund C, Söderquist B. The origin of a methicillin-resistant *Staphylococcus aureus* isolate at a neonatal ward in Sweden—Possible horizontal transfer of a staphylococcal cassette chromosome mec between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus*. *Clinical Microbiology and Infection*. 2008;**14**:1048-1056. DOI: 10.1111/j.1469-0691.2008.02090.x
- [22] Oliveira DC, Milheirico C, de Lencastre H. Redefining a structural variant of staphylococcal cassette chromosome mec, SCCmec type VI. *Antimicrobial Agents and Chemotherapy*. 2006;**50**(10):3457-3459. DOI: 10.1128/AAC.00629-06
- [23] Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*. 2003;**47**:3926-3934. DOI: 10.1128/AAC.47.12.3926-3934.2003
- [24] Memmi G, Filipe SR, Pinho MG, Fu Z, Cheung A. *Staphylococcus aureus* PBP 4 is essential for  $\beta$ -lactam resistance in community-acquired methicillin-resistant strains. *Antimicrobial Agents and Chemotherapy*. 2008;**52**:3955-3966. DOI: 10.1128/AAC.00049-08
- [25] Hososaka Y, Hanaki H, Endo H, Suzuki Y, Nagasawa Z, Otsuka Y, Nakae T, Sunakawa K. Characterization of oxacillin-susceptible mecA-positive *Staphylococcus aureus*: A new type of MRSA. *Journal of Infection and Chemotherapy*. 2007;**13**:79-86. DOI: 10.1007/s10156-006-0502-7
- [26] Belcheva A, Golemi-Kotra DA. Close-up view of the VraSR two-component system. A mediator of *Staphylococcus aureus* response to cell wall damage. *The Journal of Biological Chemistry*. 2008;**283**:12354-12364. DOI: 10.1074/jbc.M710010200
- [27] Boyle-Vavra S, Yin S, Daum RS. The VraS/VraR two-component regulatory system required for oxacillin resistance in community-acquired methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiology Letters*. 2006;**262**:163-171. DOI: 10.1111/j.1574-6968.2006.00384.x
- [28] Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, Mackenzie FM. Methicillin-resistant *Staphylococcus aureus* (MRSA): Global epidemiology and harmonisation of typing methods. *International Journal of Antimicrobial Agents*. 2012;**39**(4):273-282. DOI: 10.1016/j.ijantimicag.2011.09.030
- [29] Gubbay JB, Gosbell IB, Barbagiannakos T, Vickery AM, Mercer JL, Watson M. Clinical features, epidemiology, antimicrobial resistance, and exotoxin genes (including that

- of Pantone-Valentine leukocidin) of gentamicin-susceptible methicillin-resistant *Staphylococcus aureus* (GS-MRSA) isolated at a paediatric teaching hospital in New South Wales, Australia. *Pathology*. 2008;**40**:64-71. DOI: 10.1080/00313020701716276
- [30] Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, O'Brien FG, Tenover FC, McDougal LK, Monk AB, Enright MC. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. *Lancet*. 2005;**365**:1256-1258. DOI: 10.1016/S0140-6736(05)74814-5
- [31] Takano T, Higuchi W, Otsuka T, Baranovich T, Enany S, Saito K, Isobe H, Dohmae S, Ozaki K, Takano M, Iwao Y, Shibuya M, Okubo T, Yabe S, Shi D, Reva I, Teng LJ, Yamamoto T. Novel characteristics of community-acquired methicillin-resistant *Staphylococcus aureus* belonging to multilocus sequence type 59 in Taiwan. *Antimicrobial Agents and Chemotherapy*. 2008;**52**:837-845. DOI: 10.1128/AAC.01001-07
- [32] Shibuya Y, Hara M, Higuchi W, Takano T, Iwao Y, Yamamoto T. Emergence of the community-acquired methicillin-resistant *Staphylococcus aureus* USA300 clone in Japan. *Journal of Infection and Chemotherapy*. 2008;**14**:439-441. DOI: 10.1007/s10156-008-0640-1
- [33] Monecke S, Ehrlich R, Slickers P, Tan HL, Coombs G. The molecular epidemiology and evolution of Pantone-valentine leukocidin-positive, methicillin resistant *Staphylococcus aureus* strain USA300 in Western Australia. *Clinical Microbiology and Infection*. 2009;**15**:770-776. DOI: 10.1111/j.1469-0691.2009.02792.x
- [34] Chuang YY, Huang YC. Livestock-associated methicillin-resistant *Staphylococcus aureus* in Asia: An emerging issue? *International Journal of Antimicrobial Agents*. 2015;**45**: 334-340. DOI: 10.1016/j.ijantimicag.2014.12.007
- [35] Molla B, Byrne M, Abley M, Mathews J, Jackson CR, Fedorka-Cray P, Sreevatsan S, Wang P, Gebreyes WA. Epidemiology and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* strains of porcine origin. *Journal of Clinical Microbiology*. 2012;**50**:3687-3693. DOI: 10.1128/JCM.01971-12
- [36] Grundmann H, Schouls LM, Aanensen DM, Pluister GN, Tami A, Chlebowicz M, Glasner C, Sabat AJ, Weist K, Heuer O, Friedrich AWESCMID. Study group on molecular epidemiological markers. European staphylococcal reference laboratory working group the dynamic changes of dominant clones of *Staphylococcus aureus* causing bloodstream infections in the European region: Results of a second structured survey. *Euro Surveillance*. 2014;**19**. DOI: 10.2807/1560-7917.ES2014.19.49.20987
- [37] Hooper DC. Fluoroquinolone resistance among Gram-positive cocci. *The Lancet Infectious Diseases*. 2002;**2**:530-538. DOI: 10.1016/S1473-3099(02)00369-9
- [38] Ng EY, Trucksis M, Hooper DC. Quinolone resistance mutations in topoisomerase IV: Relationship to the flqA locus and genetic evidence that topoisomerase IV is the primary target and DNA gyrase is the secondary target of fluoroquinolones in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*. 1996;**40**:1881-1888
- [39] Ng EY, Trucksis M, Hooper DC. Quinolone resistance mediated by norA: Physiologic characterization and relationship to flqB, a quinolone resistance locus on the *Staphylococcus aureus* chromosome. *Antimicrobial Agents and Chemotherapy*. 1994;**38**:1345-1355

- [40] Howden BP, Davies JK, Johnson PDR, et al. Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: Resistance mechanisms, laboratory detection, and clinical implications. *Clinical Microbiology Reviews*. 2010;**23**(1):99-139. DOI: 10.1128/CMR.00042-09
- [41] Boyle-Vavra S, Carey RB, Daum RS. Development of vancomycin and lysostaphin resistance in a methicillin-resistant *Staphylococcus aureus* isolate. *The Journal of Antimicrobial Chemotherapy*. 2001;**48**(5):617-625
- [42] Howden BP, Johnson PD, Ward PB, Stinear TP, Davies JK. Isolates with low-level vancomycin resistance associated with persistent methicillin-resistant *Staphylococcus aureus* bacteremia. *Antimicrobial Agents and Chemotherapy*. 2006;**50**(9):3039-3047. DOI: 10.1128/AAC.00422-06
- [43] Cui L, Ma X, Sato K, Okuma K, Tenover FC, Mamizuka EM, Gemmell CG, Kim MN, Ploy MC, El-Solh N, Ferraz V, Hiramatsu K. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. *Journal of Clinical Microbiology*. 2003;**41**(1):5-14. DOI: 10.1128/JCM.41.1.5-14.2003
- [44] Herbert S, Bera A, Nerz C, Kraus D, Peschel A, Goerke C, Meehl M, Cheung A, Gotz F. Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. *PLoS Pathogens*. 2007;**3**(7):e102. DOI: 10.1371/journal.ppat.0030102
- [45] Howden BP, Smith DJ, Mansell A, Johnson PD, Ward PB, Stinear TP, Davies JK. Different bacterial gene expression patterns and attenuated host immune responses are associated with the evolution of low-level vancomycin resistance during persistent methicillin-resistant *Staphylococcus aureus* bacteraemia. *BMC Microbiology*. 2008;**8**(1):1. DOI: 10.1186/1471-2180-8-39
- [46] Matsuo M, Hishinuma T, Katayama Y, Cui L, Kapi M, Hiramatsu K. Mutation of RNA polymerase beta subunit (rpoB) promotes hVISA to-VISA phenotypic conversion of strain Mu3. *Antimicrobial Agents and Chemotherapy*. 2011;**55**(9):4188-4195. DOI: 10.1128/AAC.00398-11
- [47] Arthur M, Molinas C, Depardieu F, Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in enterococcus faecium BM4147. *Journal of Bacteriology*. 1993;**175**(1):117-127
- [48] Périchon B, Courvalin P. VanA-type vancomycin-resistant *Staphylococcus aureus*. *Antimicrobial Agents Chemother*. 2009;**53**(11):4580-4587. DOI: 10.1128/AAC.00346-09
- [49] Bien J, Sokolova O, Bozko P. Characterization of virulence Factors of *Staphylococcus aureus*: Novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response. *Journal of Pathogens*. 2011;Article ID 601905. DOI: 10.4061/2011/601905
- [50] Foster TJ, Geoghegan JA, Ganesh VK, Hook M. Adhesion, invasion and evasion: The many functions of the surface proteins of *Staphylococcus aureus*. *Nature Reviews. Microbiology*. 2014;**12**:49-62

- [51] Bowden MG, Visai L, Longshaw CM, Holland KT, Speziale P, Hook M. Is the GehD lipase from *Staphylococcus epidermidis* a collagen binding adhesin? The Journal of Biological Chemistry. 2002;**277**:43017-43023
- [52] Jan-Roblero J, Rodriguez-Martinez S, Cancino-Diaz ME, Candino-Diaz JC. Staphylococcus biofilms. In: Dhanasekaran D, Thajuddin N, editors. Microbial Biofilms—Importance and Applications. Rijeka: InTech; 2016. DOI: 10.5772/62910.ch11
- [53] Paharik AE, Horswill AR. The staphylococcal biofilm: Adhesins, regulation, and host response. Microbiology Spectrum. 2016;**4**(2). DOI: 10.1128/microbiolspec.VMBF-0022-2015
- [54] Xue T, You Y, Shang F, Sun B. Rot and Agr system modulate fibrinogen-binding ability mainly by regulating clfB expression in *Staphylococcus aureus* NCTC8325. Medical Microbiology and Immunology. 2012;**201**:81-92. DOI: 10.1007/s00430-011-0208-z
- [55] Geoghegan JA, Monk IR, O’Gara JP, Foster TJ. Subdomains N2N3 of fibronectin binding protein A mediate *Staphylococcus aureus* biofilm formation and adherence to fibrinogen using distinct mechanisms. Journal of Bacteriology. 2013;**195**:2675-2683. DOI: 10.1128/JB.02128-12
- [56] Herman-Bausier P, El-Kirat-Chatel S, Foster TJ, Geoghegan JA, Dufrene YF. *Staphylococcus aureus* fibronectin-binding protein A mediates cell-cell adhesion through low-affinity homophilic bonds. MBio. 2015;**6**. DOI: 10.1128/mBio.00413-15
- [57] Hammer ND, Skaar EP. Molecular mechanisms of *Staphylococcus aureus* iron acquisition. Annual Review of Microbiology. 2011;**65**:129-147. DOI: 10.1146/annurev-micro-090110-102851
- [58] O’Halloran DP, Wynne K, Geoghegan JA. Protein A is released into the *Staphylococcus aureus* culture supernatant with an unprocessed sorting signal. Infection and Immunity. 2015;**83**:1598-1609. DOI: 10.1128/IAI.03122-14
- [59] Roche FM, Meehan M, Foster TJ. The *Staphylococcus aureus* surface protein SasG and its homologues promote bacterial adherence to human desquamated nasal epithelial cells. Microbiology. 2003;**149**:2759-2767. DOI: 10.1099/mic.0.26412-0
- [60] Conrady DG, Wilson JJ, Herr AB. Structural basis for Zn<sup>2+</sup>-dependent intercellular adhesion in staphylococcal biofilms. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**:E202-E211. DOI: 10.1073/pnas.1208134110
- [61] Heilmann C. Adhesion mechanisms of staphylococci. Advances in Experimental Medicine and Biology. 2011;**715**:105-123. DOI: 10.1007/978-94-007-0940-9\_7
- [62] McCarthy H, Rudkin JK, Black NS, Gallagher L, O’Neill E, O’Gara JP. Methicillin resistance and the biofilm phenotype in *Staphylococcus aureus*. Frontiers in Cellular and Infection Microbiology. 2015;**5**:1. DOI: 10.3389/fcimb.2015.00001
- [63] del Pozo JL, Patel R. The challenge of treating biofilm-associated bacterial infections. Clinical Pharmacology and Therapeutics. 2007;**82**:204-209. DOI: 10.1038/sj.clpt.6100247
- [64] Lister JL, Horswill AR. *Staphylococcus aureus* biofilms: Recent developments in biofilm dispersal. Frontiers in Cellular and Infection Microbiology. 2014;**4**:178. DOI: 10.3389/fcimb.2014.00178



- [65] Reffuveille F, Josse J, Valle Q, Mongaret C, Gangloff SC. *Staphylococcus aureus* biofilms and their impact on the medical field. In: Crotty Alexander LE, editor. The Rise of Virulence and Antibiotic Resistance in *Staphylococcus aureus*. Rijeka: InTech; 2017. DOI: 10.5772/66380.ch11
- [66] Kaplan JB. Biofilm dispersal: Mechanisms, clinical implications, and potential therapeutic uses. *Journal of Dental Research*. 2010;**89**(3):205-218. DOI: 10.1177/0022034509359403
- [67] Speziale P, Pietrocola G, Foster TJ, Geoghegan JA. Protein-based biofilm matrices in staphylococci. *Frontiers in Cellular and Infection Microbiology*. 2014;**4**:171. DOI: 10.3389/fcimb.2014.00171
- [68] Foulston L, Elsholz AK, DeFrancesco AS, Losick R. The extracellular matrix of *Staphylococcus aureus* biofilms comprises cytoplasmic proteins that associate with the cell surface in response to decreasing pH. *MBio*. 2014;**5**:e01667-e01614
- [69] Scherr TD, Heim CE, Morrison JM, Kielian T. Hiding in plain sight: Interplay between staphylococcal biofilms and host immunity. *Frontiers in Immunology*. 2014;**5**:37. DOI: 10.3389/fimmu.2014.00037
- [70] Zhou G, Yuan J, Gao H. Regulation of biofilm formation by BpfA, BpfD, and BpfG in *Shewanella oneidensis*. *Frontiers in Microbiology*. 2015;**6**:790. DOI: 10.3389/fmicb.2015.00790
- [71] Zhao L, Xue T, Shang F, Sun H, Sun B. *Staphylococcus aureus* AI-2 quorum sensing associates with the KdpDE two-component system to regulate capsular polysaccharide synthesis and virulence. *Infection and Immunity*. 2010;**78**(8):3506-3515. DOI: 10.1128/IAI.00131-10
- [72] Liu Q, Yeo W-S, Bae T. The SaeRS two-component system of *Staphylococcus aureus*. J. Wing H, ed. *Genes*. 2016;**7**(10):81. DOI: 10.3390/genes7100081
- [73] Singh R, Ray P, Das A, Sharma M. Enhanced production of exopolysaccharide matrix and biofilm by a menadione-auxotrophic *Staphylococcus aureus* small-colony variant. *Journal of Medical Microbiology*. 2010;**59**(Pt 5):521-527. DOI: 10.1099/jmm.0.017046-0
- [74] Vandenesch F, Lina G, Henry T. *Staphylococcus aureus* Hemolysins, bi-component leukocidins, and cytolytic peptides: A redundant arsenal of membrane-damaging virulence factors? *Frontiers in Cellular and Infection Microbiology*. 2012;**2**:12. DOI: 10.3389/fcimb.2012.00012
- [75] Velhne M, Kozoderović G, Suvajdžić L, Todorović D, Petrović J. Genetic mechanisms of methicillin resistance in *Staphylococcus aureus* (Mrsa) and its capacity of transmission: A brief review. *Arhiv Veterinarske Medicine*. 2016;**9**(2):29-37
- [76] Verdon J, Girardin N, Lacombe C, Berjeaud JM, Hechard Y. Delta-hemolysin, an update on a membrane-interacting peptide. *Peptides*. 2009;**30**:817-823. DOI: 10.1016/j.peptides.2008.12.017
- [77] Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, Deleo FR, Otto M. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nature Medicine*. 2007;**13**:1510-1514. DOI: 10.1038/nm1656



- [78] Chatterjee SS, Chen L, Joo H-S, Cheung GYC, Kreiswirth BN, Otto M. Distribution and regulation of the mobile genetic element-encoded phenol-soluble Modulins PSM-mec in methicillin-resistant *Staphylococcus aureus*. Horsburgh MJ, ed. PLoS One. 2011; 6(12):e28781. DOI: 10.1371/journal.pone.0028781
- [79] Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, O'Neill G, Day NP. Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. Infection and Immunity. 2002;70(9):4987-4996. DOI: 10.1128/IAI.70.9.4987-4996.2002
- [80] Watkins RR, David MZ, Salata RA. Current concepts on the virulence mechanisms of methicillin-resistant *Staphylococcus aureus*. Journal of Medical Microbiology. 2012;61(Pt 9): 1179-1193. DOI: 10.1099/jmm.0.043513-0
- [81] Dumitrescu O, Boisset S, Badiou C, Bes M, Benito Y, Reverdy ME, Vandenesch F, Etienne J, Lina G. Effect of antibiotics on *Staphylococcus aureus* producing Panton-Valentine leukocidin. Antimicrobial Agents and Chemotherapy. 2007;51:1515-1519. DOI: 10.1128/AAC.01201-06
- [82] Stevens DL, Ma Y, Salmi DB, McIndoo E, Wallace RJ, Bryant AE. Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. The Journal of Infectious Diseases. 2007;195:202-211. DOI: 10.1086/510396
- [83] Schubert S, Schwertz H, Weyrich AS, Franks ZG, Lindemann S, Otto M, Behr H, Loppnow H, Schlitt A, Russ M, Presek P, Werdan K, Buerke M. *Staphylococcus aureus*  $\alpha$ -toxin triggers the synthesis of B-cell lymphoma 3 by human platelets. Toxins (Basel). 2011;3:120-133. DOI: 10.3390/toxins3020120
- [84] Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, Sensabaugh GF, Perdreau-Remington F. Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. Lancet. 2006;367:731-739. DOI: 10.1016/S0140-6736(06)68231-7
- [85] Dunman PM, Murphy E, Haney S, Palacios D, Tucker-Kellogg G, Wu S, Brown EL, Zagursky RJ, Shlaes D, Projan SJ. Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the agr and/or sarA loci. Journal of Bacteriology. 2001;183:7341-7353. DOI: 10.1128/JB.183.24.7341-7353.2001
- [86] Thurlow LR, Hanke ML, Fritz T, Angle A, Aldrich A, Williams SH, Engebretsen IL, Bayles KW, Horswill AR, Kielian T. *Staphylococcus aureus* biofilms prevent macrophage phagocytosis and attenuate inflammation in vivo. Journal of Immunology (Baltimore, Md.: 1950). 2011;186(11):6585-6596. DOI: 10.4049/jimmunol.1002794
- [87] Nair SP, Bischoff M, Senn MM, Berger-Bachi B. The sigmaB regulon influences internalization of *Staphylococcus aureus* by osteoblasts. Infection and Immunity. 2003;71:4167-4170. DOI: 10.1128/IAI.71.7.4167-70.2003
- [88] Zielinska AK, Beenken KE, Joo HS, Mrak LN, Griffin LM, Luong TT, Lee CY, Otto M, Shaw LN, Smeltz MS. Defining the strain-dependent impact of the staphylococcal accessory regulator (sarA) on the alpha-toxin phenotype of *Staphylococcus aureus*. Journal of Bacteriology. 2011;193:2948-2958. DOI: 10.1128/JB.01517-10